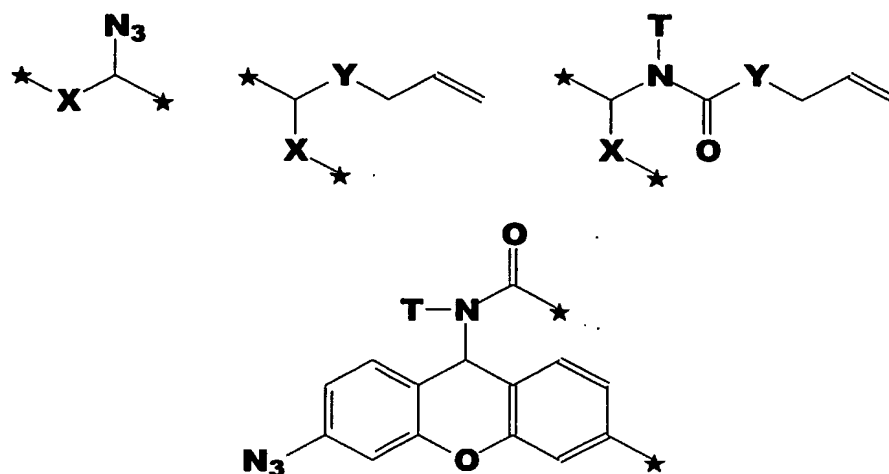


CLAIMS

1. A nucleotide or nucleoside having a base attached to a detectable label via a cleavable linker, characterised in that the cleavable linker contains a moiety selected from the group comprising:



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- (wherein X is selected from the group comprising O, S, NH and NQ wherein Q is a C₁₋₁₀ substituted or unsubstituted alkyl group, Y is selected from the group comprising O, S, NH and N(allyl), T is hydrogen or a C₁₋₁₀ substituted or unsubstituted alkyl group and * indicates where the moiety is connected to the remainder of the nucleotide or nucleoside).

2. The nucleotide or nucleoside as claimed in claim 1 wherein X is O or S.

3. The nucleotide or nucleoside as claimed in claim 1 or claim 2 wherein Y is O or S.

4. The nucleotide or nucleoside as claimed in any one of claims 1 to 3 wherein Y is O.

5. The nucleotide or nucleoside as claimed in any

one preceding claim wherein the moiety may be present in the nucleotide or nucleoside in either of two orientations.

5 6. The nucleotide or nucleoside as claimed in any one preceding claim wherein the base is a purine, or a pyrimidine.

10 7. The nucleotide or nucleoside as claimed in any one preceding claim wherein the linker is attached to the 5-position of a pyrimidine or 7-position of a purine.

15 8. The nucleotide or nucleoside as claimed in any one preceding claim wherein the base is a deazapurine.

20 9. The nucleotide or nucleoside as claimed in any one preceding claim wherein the nucleotide has a ribose or deoxyribose sugar moiety.

25 10. The nucleotide or nucleoside as claimed in claim 9 wherein the ribose or deoxyribose sugar comprises a hydroxyl protecting group attached to the 2' or 3' oxygen atom.

30 11. The nucleotide or nucleoside as claimed in claim 10 wherein the same chemical conditions may be used to effect cleavage of the cleavable linker and to remove the hydroxyl protecting group.

35 12. The nucleotide or nucleoside as claimed in any one preceding claim wherein the nucleotide is a deoxyribonucleotide triphosphate.

13. The nucleotide or nucleoside as claimed in any

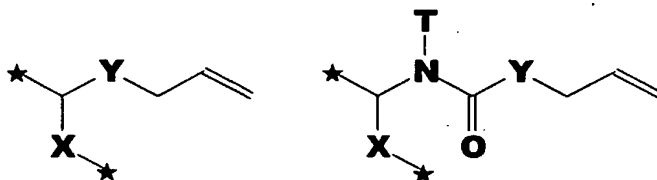
one preceding claim wherein the detectable label is a fluorophore.

14. An oligonucleotide comprising one or more
5 nucleotides as defined in any one of claims 1 to 13.

15. The oligonucleotide as claimed in claim 14 wherein at least one nucleotide is present at a terminal position in said oligonucleotide.

10

16. A method of cleaving a linker that contains a moiety selected from the groups comprising:



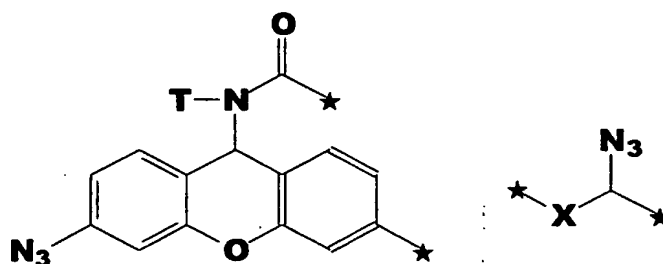
15

(wherein X is selected from the group comprising O, S, NH and NQ wherein Q is a C₁₋₁₀ substituted or unsubstituted alkyl group, Y is selected from the group comprising O, S, NH and N(allyl), T is hydrogen or a C₁₋₁₀ substituted or unsubstituted alkyl group and
20 * indicates where the moiety is connected to the remainder of a nucleotide or nucleoside), said linker being present in a nucleotide or nucleoside and connecting the base thereof to a detectable label, said method comprising contacting
25 the nucleotide or nucleoside with a water-soluble phosphine-based transition metal catalyst.

17. The method as claimed in claim 16 wherein the
30 transition metal is selected from the group comprising platinum, palladium, rhodium, ruthenium, osmium and iridium.

18. The method as claimed in claim 16 wherein the transition metal is palladium.

19. A method of cleaving a linker that contains a moiety selected from the groups comprising:



(wherein X is selected from the group comprising O, S, NH and NQ wherein Q is a C₁₋₁₀ substituted or unsubstituted alkyl group, T is hydrogen or a C₁₋₁₀ substituted or unsubstituted alkyl group and * indicates where the moiety is connected to the remainder of a nucleotide or nucleoside),

15 said linker being present in a nucleotide or nucleoside and connecting the base thereof to a detectable label, said method comprising contacting the nucleotide or nucleoside with a water-soluble phosphine.

20

20. The method as claimed in any one of claims 16 to 19 wherein said phosphine is a derivatised triaryl phosphine or a derivatised trialkyl phosphine.

25 21. The method as claimed in any one of claims 16 to 20 wherein said phosphine is a triaryl phosphine derivatised with one or more functionalities selected from the group comprising amino, hydroxyl, carboxyl and sulfonate.

30

22. The method as claimed in any one of claims 16 to

21 wherein the water-soluble phosphine is selected from the group comprising 3,3',3"-phosphinidynetris (benzenesulfonic acid) or tris(2-carboxyethyl)phosphine and their salts.

5

23. The method as claimed in any one of claims 16 to 19 wherein said phosphine contains one or more nitrogen atoms.

10

24. The method as claimed in any one of claims 16 to 23 wherein X is O or S.

25. The method as claimed in any one of claims 16 to 24 wherein Y is O or S.

15

26. The method as claimed in any one of claims 16 to 25 wherein Y is O.

20

27. The method as claimed in any one of claims 16 to 26 wherein the moieties may be present in the nucleoside or nucleotide in either of two orientations.

25

28. The method of in any one of claims 16 to 27 wherein said label is detected before said linker is cleaved.

30

29. The method of claim 28 wherein said method involves cleavage of the linker in a nucleotide which is incorporated into an oligonucleotide.

35

30. The method of claim 29 wherein said incorporated nucleotide is present at a terminal position in said oligonucleotide.

31. The method as claimed in any one of claims 16 to

30 wherein the base is a purine, or a pyrimidine.

32. The method of claim 31, wherein the linker is attached to the 5-position of a pyrimidine or 7-
5 position of a purine.

33. The method as claimed in any one of claims 16 to 32 wherein the base is a deazapurine.

10 34. The method as claimed in any one of claims 16 to 33 wherein the nucleotide has a ribose or deoxyribose sugar moiety.

15 35. The method as claimed in claim 34 wherein the ribose or deoxyribose sugar comprises a hydroxyl protecting group attached to the 2' or 3' oxygen atom.

36. The method as claimed in any one of claims 16 to 35 wherein the nucleotide is a deoxyribonucleotide
20 triphosphate.

37. The method as claimed in any one of claims 16 to 36 wherein the detectable label is a fluorophore.

25 38. The method as claimed in any one of claims 29 to 37 wherein the incorporating step is effected by a reverse transcriptase, a terminal transferase or a polymerase.

30 39. The method of claim 38 wherein the polymerase is a *Thermococcus* sp.

40. The method of claim 39 wherein the *Thermococcus* sp is 9°N or a single mutant or double mutant thereof.

35 41. The method of claim 40 wherein the double mutant

is -Y409V A485L.

42. The method as claimed in any one of claims 29 to
41 wherein the detectable label and/or the cleavable
5 linker is of a size sufficient to prevent the
incorporation of a subsequent nucleotide into the
nascent oligonucleotide.

43. The method as claimed in any one of claims 29 to
10 42 wherein the incorporated nucleotide contains a 3'OH
blocking group which serves to prevent incorporation
of any further nucleotides.

44. The method as claimed in claim 43 wherein the
15 same chemical conditions used to effect cleavage of
the cleavable linker serve to remove the 3'OH blocking
group.

45. The method as claimed in any one of claims 29 to
20 44 wherein the detecting step permits the
identification of the incorporated nucleotide.

46. A method for determining the identity of a
nucleotide in a target single-stranded polynucleotide,
25 comprising:

(a) providing one or more of the nucleotides A,
G, C and T or U in which each of said nucleotides has
a base that is attached to a distinct detectable label
via a linker, said linker being cleavable with a
30 water-soluble phosphine; and a nascent polynucleotide
complementary to the target polynucleotide, one of
said provided nucleotides being suitable for
incorporation into said nascent polynucleotide;

(b) incorporating the nucleotide suitable for
35 incorporation into said nascent polynucleotide; and

(c) carrying out a method as defined in claim 45.

47. The method as claimed in claim 46 wherein steps (a) and (b) are repeated one or more times so as to determine the identity of a plurality of bases in the target polynucleotide.

5

48. A method as claimed in claim 46 or claim 47 wherein step (a) comprises contacting the provided nucleotides with the target sequentially.

10 49. A method as claimed in any one of claims 46 to 48 wherein step (a) comprises at least one substep of providing one of the four said nucleotides.

15 50. A method as claimed in claim 49 wherein step (a) further comprises, after said substep, providing the other three nucleotides simultaneously or sequentially.

20 51. A method as claimed in claim 50 wherein said other three nucleotides are added sequentially, either by providing them one at a time; or two simultaneously and then the remaining one; or one of the three and then the remaining two simultaneously.

25 52. A method as claimed in any one of claims 46 to 48 wherein step (a) comprises at least a substep of providing two of the four said nucleotides.

30 53. A method as claimed in claim 52 wherein step (a) further comprises, after said substep, providing the other two nucleotides simultaneously or sequentially.

35 54. A method as claimed in any one of claims 46 to 48 wherein step (a) comprises at least a substep of providing three of the four said nucleotides.

55. A method as claimed in claim 54 wherein step (a) further comprises, after said substep, providing the remaining nucleotide of the four said nucleotides.
- 5 56. A method as claimed in any one of claims 46 to 48 wherein step (a) comprises providing all four of the said nucleotides and contacting them with the target simultaneously.
- 10 57. A method as claimed in any one of claims 46 to 56 wherein any unincorporated nucleotides are removed prior to the provision of further nucleotide(s) and/or the effecting of step (c).
- 15 58. A method as claimed in claim 57 wherein step (c) is effected without unsuitable nucleotides having been provided after provision of said suitable nucleotide.
59. Use of a nucleotide as defined in any one of
20 claims 1 to 13 in a Sanger or Sanger-type sequencing method.
60. A method of using a nucleotide of claims 1
25 wherein said method includes a Sanger or Sanger-type sequencing method.